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DB Name	Query	Hit Count	Set Name
USPT	l30 and l27	0	L31
USPT	scfv	255	L30
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USPT	l17 same (both or combinantion or combined)	1074	L26
USPT	l24 and fibronectin	0	L25
USPT	l23 and l22	9	L24
USPT	bismuth212 or bismuth-212 or bismuth213 or bismuth-213 or astatine or astatine211 or astatine-211	290	L23
USPT	imaging.ti. and (alpha or beta)	1755	L22
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USPT	l16 or l18	175	L20
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USPT	immunoscint$.ti.	1	L15
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USPT	l12 and tumor$	33	L13
USPT	occlusion same (radioact$ or radionucleo$)	69	L12
USPT	l9 and (edb or ed-b)	0	L11
USPT	l9 and (fibronectin or bc-1)	0	L10
USPT	e6 same tin	13	L9
USPT	l6 and cy7	0	L8
USPT	l6 and photosen$	0	L7
USPT	l1 and fibronectin and bc-1 and coagulant and radioactive	3	L6
USPT	l4 and coagulant	3	L5
USPT	l2 and bc-1	3	L4
USPT	l2 and be-1	0	L3
USPT	l1 and fibronectin	3	L2
USPT	(6036955 or 6004555 or 6093399).pn.	3	L1

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☐ 1. Document ID: WO 9745544 A1

L7: Entry 1 of 3 File: EPAB Dec 4, 1997

PUB-NO: WO009745544A1
DOCUMENT-IDENTIFIER: WO 9745544 A1
TITLE: ANTIBODIES TO THE ED-B DOMAIN OF FIBRONECTIN, THEIR CONSTRUCTION AND USES

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: WO 9623816 A1

L7: Entry 2 of 3 File: DWPI Aug 8, 1996

DERWENT-ACC-NO: 1996-371376
DERWENT-WEEK: 199637
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TITLE: Modified binding protein fused to casein kinase II enzyme substrate - can be radio-labelled to treat patients in need of radiotherapy, and for use in analytical applications

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 3. Document ID: DE 69516403 E, WO 9532001 A1, AU 9525662 A, EP 760679 A1, IT 1268802 B, US 5869051 A, EP 760679 B1

L7: Entry 3 of 3 File: DWPI May 25, 2000

DERWENT-ACC-NO: 1996-020358
DERWENT-WEEK: 200032
COPYRIGHT 2000 DERWENT INFORMATION LTD

TITLE: Conjugates of carrier molecule and organic cpd. able to produce singlet oxygen - are useful against viral, fungal and bacterial infections, and against tumours

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Clip Img | Image

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Terms	Documents
('EP 760679B' 'WO 9623816A' 'WO009745544A1')[ABPN1,WKU]	3

Display 50 Documents, starting with Document: 3

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L4: Entry 1 of 4 File: USPT Jul 25, 2000

US-PAT-NO: 6093399
DOCUMENT-IDENTIFIER: US 6093399 A

TITLE: Methods and compositions for the specific coagulation of vasculature

DATE-ISSUED: July 25, 2000

INT-CL: [7] A61K 39/395
US-CL-ISSUED: 424/182; 530/387.3, 530/387.7, 530/387.9, 530/387.1, 530/388.1, 530/388.22, 530/388.85, 530/391.7, 530/391.9, 424/178.1, 424/180.1, 424/179.1
US-CL-CURRENT: 424/182.1; 424/178.1, 424/179.1, 424/180.1, 530/387.1, 530/387.3, 530/387.7, 530/387.9, 530/388.1, 530/388.22, 530/388.85, 530/391.7, 530/391.9
FIELD-OF-SEARCH: 424/182.1, 424/178.1, 424/179.1, 424/180.1, 530/387.1, 530/387.3, 530/387.7, 530/387.9, 530/388.1, 530/388.22, 530/388.85, 530/391.7, 530/391.9

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L4: Entry 2 of 4 File: USPT Mar 14, 2000

US-PAT-NO: 6036955
DOCUMENT-IDENTIFIER: US 6036955 A

TITLE: Kits and methods for the specific coagulation of vasculature

DATE-ISSUED: March 14, 2000

INT-CL: [7] A61K 39/395
US-CL-ISSUED: 424/136.1; 424/130.1, 424/141.1, 424/143.1, 424/144.1, 424/145.1, 424/85.2, 424/152.1, 424/155.1, 424/156.1, 424/158.1, 424/178.1, 530/8, 530/387.3, 530/387.7, 530/388.7, 530/389.6, 530/389.7, 530/391.7
US-CL-CURRENT: 424/136.1; 424/130.1, 424/141.1, 424/143.1, 424/144.1, 424/145.1, 424/152.1, 424/155.1, 424/156.1, 424/158.1, 424/178.1, 424/85.2, 514/8, 530/387.3, 530/387.7, 530/388.7, 530/389.6, 530/389.7, 530/391.7
FIELD-OF-SEARCH: 424/136.1, 424/138.1, 424/141.1, 424/143.1, 424/144.1, 424/145.1, 424/85.2, 424/152.1, 424/155.1, 424/156.1, 424/158.1, 424/178.1, 530/8, 530/387.3, 530/387.7, 530/388.7, 530/388.8, 530/389.6, 530/389.7, 530/391.7

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L4: Entry 3 of 4 File: USPT Dec 21, 1999

US-PAT-NO: 6004555
DOCUMENT-IDENTIFIER: US 6004555 A

TITLE: Methods for the specific coagulation of vasculature

DATE-ISSUED: December 21, 1999

INT-CL: [6] A61K 39/395, A61K 35/14, C07K 16/00, G01N 33/574
US-CL-ISSUED: 424/181.1; 424/178.1, 424/180.1, 530/381, 530/382, 530/383, 530/384, 530/391.7, 435/7.23
US-CL-CURRENT: 424/181.1; 424/178.1, 424/180.1, 435/7.23, 530/381, 530/382, 530/383, 530/384, 530/391.7
FIELD-OF-SEARCH: 424/178.1, 424/180.1, 424/181.1, 530/381, 530/382, 530/383, 530/384, 530/391.7

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L4: Entry 4 of 4 File: USPT Mar 2, 1999

US-PAT-NO: 5877289
DOCUMENT-IDENTIFIER: US 5877289 A

TITLE: Tissue factor compositions and ligands for the specific coagulation of vasculature

DATE-ISSUED: March 2, 1999

INT-CL: [6] A61K 39/395
US-CL-ISSUED: 530/387.1; 530/387.3, 530/387.7, 530/387.9, 530/388.1, 530/388.22, 530/388.85, 530/391.7, 530/391.9, 530/381
US-CL-CURRENT: 530/387.1; 530/381, 530/387.3, 530/387.7, 530/387.9, 530/388.1, 530/388.22, 530/388.85, 530/391.7, 530/391.9
FIELD-OF-SEARCH: 424/136.1, 424/135.1, 424/134.1, 424/69.7, 514/2, 514/12, 514/834, 530/387.3, 530/350, 530/381, 530/387.1, 530/387.7, 530/387.9, 530/388.1, 530/388.22, 530/388.85, 530/391.7, 530/391.9

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☐ 1. Document ID: US 6132730 A

L5: Entry 1 of 7 File: USPT Oct 17, 2000

DOCUMENT-IDENTIFIER: US 6132730 A
TITLE: Combined tissue factor and factor VIIa methods and compositions for coagulation and tumor treatment

BSPR:
The antibody constructs and conjugates may be operatively attached to at least a first cytotoxic or otherwise anti-cellular agent. They may also be operatively attached to at least a first coagulation factor. In attachment to coagulants, bispecific constructs may also be advantageously employed (e.g., using two antibody binding regions), although the covalent linkages are generally preferred for use with the toxins. Any one or more of the toxic or coagulating agents known in the art may be employed in such "immunotoxins" or "coaguligands", and Tissue Factor or Tissue Factor derivatives may also be employed as part of the coaguligands, where the coaguligand is the second, "anti-cancer agent".

DRPR:
FIG. 12A: Mice with 0.8 to 1.0 cm diameter C1300(Mu.gamma.) tumors were given two intravenous injections of B21-2/10H10-tTF coaguligand (.circle-solid.) spaced 6 days apart (arrows). Mice in control groups received equivalent doses of tTF alone (.quadrature.), CAMPATH-2/10H10 plus tTF (.DELTA.), or phosphate buffered saline (.smallcircle.). Mice that received B21-2/OX7 and tTF had similar tumor responses to those in

DEPR:
Despite the significant impairment of coagulative capacity of the tTF, tTF can promote blood coagulation when tethered or functionally associated by some other means with a phospholipid or membrane environment. For example, it is demonstrated herein that using a bispecific antibody that binds tTF to a plasma membrane antigen allows restoration of useful coagulating activity. This led one of the present inventors to develop methods for the specific coagulation of tumor vascular in vivo by using targeting constructs to deliver tTF or variants thereof specifically to the tumor vascular or stroma (WO 96/01653). Intravenous administration of such a "coaguligand" leads to localization of the coagulants within the tumor, thrombosis of the tumor vessels, and resultant tumor necrosis.

DEPR:
The development of the intelligent, targeted delivery of coagulants to the tumor vasculature, as exemplified using a bispecific targeting antibody-tTF composition, may be seen as representing an improvement over classic immunotoxin therapy. In fact, such coaguligand treatment induces thrombosis of tumor vessels in less than 30 minutes, in comparison to about 6 hours necessary to achieve the same effect following administration of an immunotoxin. Furthermore, there was no notable side effects as a result of the coaguligand treatment. Although the targeted delivery of a coagulant such as tTF was surprisingly effective, this stills requires the preparation of the "targeting construct".

DEPR:

The surprising finding of the inventors that tTF specifically localized within tumors sufficiently so as to cause anti-tumor effect was discovered during studies using tTF as a control in antibody-coagulant ("coaguligand") tumor targeting studies. From this initial discovery, the inventors developed the various aspects of the invention disclosed herein. The Tissue Factor compounds or constructs for use in the present invention have thus been developed from the original tTF first employed. Accordingly, various TF constructs may now be employed, including many different forms of tTF, longer but still impaired TFs, mutants TFs, any truncated, variant or mutant TFs modified or otherwise conjugated to improve their half-life, and all such functional equivalents thereof. However, it will be understood that each of the TF constructs for use in the invention are unified by the need to be "coagulation-deficient". As detailed herein below, there are various structural considerations that may be employed in the design of candidate coagulation-deficient TFs, and various assays are available for confirming that the candidate TFs are indeed suitable for use in the treatment aspects of the present invention. Given that the technological skills for creating a variety of compounds, e.g., using molecular biology, are routine to those of ordinary skill in the art, and given the extensive structural and functional guidance provided herein, the ordinary artisan will be readily able to make and use a number of different coagulation-deficient TFs in the context of the present invention.

DEPR:
In terms of surgery, any surgical intervention may be practiced in combination with the present invention. In connection with radiotherapy, any mechanism for inducing DNA damage locally within tumor cells is contemplated, such as .gamma.-irradiation, X-rays, UV-irradiation, microwaves and even electronic emissions and the like. The directed delivery of radioisotopes to tumor cells is also contemplated, and this may be used in connection with a targeting antibody or other targeting means. Cytokine therapy also has proven to be an effective partner for combined therapeutic regimens. Various cytokines may be employed in such combined approaches. Examples of cytokines include IL-1.alpha. IL-1.beta., IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, TGF-.beta., GM-CSF, M-CSF, G-CSF, TNF.alpha., TNF.beta., LAF, TCGF, BCGF, TRF, BAF, BDG, MP, LIF, OSM, TMF, PDGF, IFN-.alpha., IFN-.beta., IFN-.gamma.. Cytokines are administered according to standard regimens, consistent with clinical indications such as the condition of the patient and relative toxicity of the cytokine.

DEPR:
In certain embodiments, the present invention shows that the anti-tumor activity of tTF is enhanced when administered in combination with a chemotherapeutic agent. The mechanisms by which the drugs enhance the anti-tumor activity of tTF have not been precisely defined, but the inventors believe that the drug kills proliferating tumor cells creating necrotic areas that cause phagocytic cells to infiltrate the tumor. IL-1, TNF.alpha. and other cytokines released by the infiltrating cells then activate the tumor vascular endothelium making it better able to support coagulation by tTF, a generally weak thrombogen. The drug thus enhances the thrombotic action of tTF.

DEPR:
Any one or more of the coagulation-deficient TF constructs of the invention may be used in combination with immunotoxins (ITs) and/or coaguligands in which the targeting portion thereof (e.g., antibody or ligand) is directed to a relatively specific marker of the tumor cells, tumor vasculature or tumor stroma. In common with the chemotherapeutic agents discussed above, it is possible that the use of a coagulation-deficient TF construct in combination with a targeted toxic agent (IT) or coagulant (coaguligand) will result in the distinct agents being directed against different targets within the tumor environment. This should result in additive, markedly greater than additive or even synergistic results.

DEPR:
With coaguligands, the burden of very stringent targeting, e.g., as imposed when using immunotoxins, is lessened. Therefore, to achieve specific targeting means that coagulation is promoted in the tumor vasculature relative to the vasculature in non-tumor sites. Thus, specific targeting of a coaguligand is a functional term rather than a purely physical term relating to the biodistribution properties of the targeting agent, and it is not unlikely that useful targets may be not be entirely tumor-restricted, and that targeting ligands which are

effective to promote tumor-specific coagulation may nevertheless be found at other sites of the body following administration.

DEPR:
Another means of defining a tumor-associated target is in terms of the characteristics of the tumor cell, rather than describing the biochemical properties of an antigen expressed by the cell. Accordingly, the inventors contemplate that any antibody that preferentially binds to a tumor cell may be used as the targeting component of an immunotoxin or coaguligand. The preferential tumor cell binding is again based upon the antibody exhibiting high affinity for the tumor cell and not having significant reactivity with life-sustaining normal cells or tissues, as defined above.

DEPR:
The cells of the vasculature are intended as targets for use in the present invention. In these cases, at least one binding region of the immunotoxin or coaguligand will be capable of binding to an accessible marker preferentially expressed by disease-associated vasculature endothelial cells. The exploitation of the vascular markers is made possible due to the proximity of the vascular endothelial cells to the disease area and to the products of the local aberrant physiological processes. For example, tumor vascular endothelial cells are exposed to tumor cells and tumor-derived products that change the phenotypic profile of the endothelial cells.

DEPR:
Binding of fibrinogen to its receptor alters the conformation of the carboxyl-terminal aspects of the A.alpha.-chains, exposing the sequences which reside in the coiled-coil connector segments between the D and E domains of the molecule, generating the RIBS epitopes. In practical terms, the RIBS sequences are proposed as epitopes for use in targeting with a coaguligand. The MAbs 9F9 and 155B16 may thus be advantageously used, as may the antibodies described by Zamarron et al. (1991).

DEPR:
p-155, a multimeric platelet protein that is expressed on activated platelets (Hayward et al., 1991), may be targeted using the invention. Platelets respond to a large number of stimuli by undergoing complex biochemical and morphological changes. These changes are involved in physiological processes including adhesion, aggregation, and coagulation. Platelet activation produces membrane alterations that can be recognized by monoclonal antibodies. The monoclonal antibody JS-1 (Hayward et al., 1991) is one such antibody contemplated for use as part of a coaguligand.

DEPR:
For certain applications, it is envisioned that the second therapeutic agents will be pharmacological agents attached to antibodies or growth factors, particularly cytotoxic or otherwise anti-cellular agents having the ability to kill or suppress the growth or cell division of endothelial cells. In general, the secondary aspects of the invention contemplate the use of any pharmacological agent that can be conjugated to a targeting agent, preferably an antibody, and delivered in active form to the targeted endothelium or stroma. Exemplary anti-cellular agents include chemotherapeutic agents, radioisotopes as well as cytotoxins. In the case of chemotherapeutic agents, the inventors propose that agents such as a hormone such as a steroid; an anti-metabolite such as cytosine arabinoside, fluorouracil, methotrexate or aminopterin; an anthracycline; mitomycin C; a vinca alkaloid; demecolcine; etoposide; mithramycin; or an anti-tumor alkylating agent such as chlorambucil or melphalan, will be particularly preferred. Other embodiments may include agents such as a cytokine, growth factor, bacterial endotoxin or the lipid A moiety of bacterial endotoxin. In any event, it is proposed that agents such as these may, if desired, be successfully conjugated to a targeting agent, preferably an antibody, in a manner that will allow their targeting, internalization, release or presentation to blood components at the site of the targeted endothelial cells as required using known conjugation technology (see, e.g., Ghose et al., 1983 and Ghose et al., 1987).

DEPR:
It is contemplated that patients chosen for combined studies would have failed to respond to at least one course of conventional therapy and had to have

objectively measurable disease as determined by physical examination, laboratory techniques, or radiographic procedures. Where murine monoclonal antibody portions are employed in the immunotoxins or coaguligands, the patients should have no history of allergy to mouse immunoglobulin. Any chemotherapy should be stopped at least 2 weeks before entry into the study.

DEPR:
These Tissue Factor and IT or coaguligand combinations may be administered over a period of approximately 4-24 hours, with each patient receiving 2-4 infusions at 2-7 day intervals. Administration can also be performed by a steady rate of infusion over a 7 day period. The infusion given at any dose level should be dependent upon any toxicity observed. Hence, if Grade II toxicity was reached after any single infusion, or at a particular period of time for a steady rate infusion, further doses should be withheld or the steady rate infusion stopped unless toxicity improved. Increasing doses of Tissue Factor with either immunotoxins or coaguligands should be administered to groups of patients until approximately 60% of patients showed unacceptable Grade III or IV toxicity in any category. Doses that are 2/3 of this value could be defined as the safe dose.

DEPR:
Physical examination, tumor measurements, and laboratory tests should, of course, be performed before treatment and at intervals up to 1 month later. Laboratory tests should include complete blood counts, serum creatinine, creatine kinase, electrolytes, urea, nitrogen, SGOT, bilirubin, albumin, and total serum protein. Serum samples taken up to 60 days after treatment should be evaluated by radioimmunoassay for the presence of the intact Tissue Factor, immunotoxin and/or coaguligand or components thereof and antibodies against any portions thereof. Immunological analyses of sera, using any standard assay such as, for example, an ELISA or RIA, will allow the pharmacokinetics and clearance of the therapeutic agent to be evaluated.

DEPR:
This culturing provides a population of hybridomas from which specific hybridomas are selected. Typically, selection of hybridomas is performed by culturing the cells by single-clone dilution in microtiter plates, followed by testing the individual clonal supernatants (after about two to three weeks) for the desired reactivity. The assay should be sensitive, simple and rapid, such as radioimmunoassays, enzyme immunoassays, cytotoxicity assays, plaque assays, dot immunobinding assays, and the like.

DEPR:
A histological study was performed to determine whether intravenous administration of the B21-2/10H10-tTF coaguligand induced selective thrombosis of tumor vasculature in mice bearing subcutaneous C1300(Mu.gamma.) neuroblastomas of 0.8 to 1.0 cm diameter (FIG. 9). Within 30 minutes, all vessels throughout the tumor were thrombosed, containing occlusive platelet aggregates, packed erythrocytes, and fibrin. At this time, tumor cells were indistinguishable histologically from tumor cells of untreated mice.

DEPR:
After 4 hours, however, there were signs of tumor cell injury. The majority of tumor cells had separated from one another and had pyknotic nuclei, and the tumor interstitium commonly contained erythrocytes. By 24 hours, the tumor showed advanced necrosis, and by 72 hours, the entire central region of the tumor had condensed into amorphous debris. These studies indicated that the predominant occlusive effect of the B21-2/10H10-tTF coaguligand on tumor vessels is mediated through binding to Class II antigens on tumor vascular endothelium.

DEPR:
Coaguligand treatment was well tolerated, mice lost no weight and retained normal appearance and activity levels. At the treatment dose of 0.6 mg/kg B21-2/10H10 plus 0.5 mg/kg tTF, toxicity was observed in only two of forty mice (thrombosis of tail vein). It is important to note that neither thrombi, nor histological or morphological abnormalities were visible in paraffin sections of liver, kidney, lung, intestine, heart, brain, adrenals, pancreas, or spleen from the tumor-bearing mice 30 minutes or 24 hours after administration of coaguligand or free tTF. Furthermore, no signs of toxicity (behavioral changes, physical signs, weight changes) were observed in treated animals.

DEPR:
The inventors next investigated whether intravenous administration of the B21-2/10H10-tTF coaguligand could inhibit the growth of large (0.8 to 1.0 cm diameter) tumors in mice. The pooled results from three separate studies indicate that mice receiving B21-2/10H10-tTF coaguligand had complete tumor regressions lasting four months or more. These anti-tumor effects were significantly greater than for all other treatment groups (FIG. 12A).

DEPR:
Surprisingly, the inventors found that the anti-tumor effect of the B21-2/10H10-tTF coaguligand was attributable, in part, to a non-targeted effect of tTF. Tumors in mice receiving tTF alone or mixed with control bispecific antibodies (CAMPATH II/10H10 or B21-2/OX7) grew significantly more slowly than tumors in mice receiving antibodies or saline alone (FIG. 12A; FIG. 12B).

DEPR:
In animals that did not show complete tumor regression after B21-2/10H10-tTF coaguligand treatment, the tumors grew back from a surviving microscopic rim of cells at the periphery of the tumor. Immunohistochemical examination of these tumors revealed that the vascular endothelium at the invading edge of the tumors lacked detectable Class II antigens, consistent with a lack of thrombosis of these vessels by the coaguligand permitting local tumor cell survival. Thus, coadministration of a drug acting on the tumor cells themselves would likely improve efficacy, as has been observed with another antivascular therapy (Burrows and Thorpe, 1992; Burrows and Thorpe 1993; Burrows and Thorpe 1994; U.S. Ser. Nos. 07/846,349; 08/205,330; 08/295,868; and 08/350,212).

DEPR:
The inventors previously demonstrated that a powerfully cytotoxic ricin A-chain immunotoxin directed against the tumor cells themselves was virtually devoid of anti-tumor activity when administered to mice with large C1300(Mu.gamma.) tumors (Burrows and Thorpe, 1993; U.S. Ser. Nos. 07/846,349; 08/205,330; 08/295,868; and 08/350,212). The lack of activity was due to the inability of the immunotoxin to gain access to tumor cells in large tumor masses, thus attesting to the comparative effectiveness of coaguligand therapy.

DEPR:
The studies using coaguligands confirm the therapeutic potential of selective initiation of the blood coagulation cascade in tumor vasculature (U.S. Ser. Nos. 08/273,567; 08/482,369; 08/485,482; 08/487,427; 08/479,733; 08/472,631; 08/479,727; and 08/481,904). The induction of tumor infarction by targeting a thrombogen to tumor endothelial cell markers is therefore an effective anti-cancer strategy and may even result in the eradication of primary solid tumors and vascularized metastases.

DEPL:
E2. Immunotoxin and Coaguligand Combinations and Therapy

DEPV:
Wawrzynczak and Thorpe In: Immunoconjugates: Antibody Conjugates in Radioimaging and Therapy of Cancer, Vogel (ed.), New York, Oxford University Press, pp. 28-55, 1987.

☐ 2. Document ID: US 6132729 A

L5: Entry 2 of 7 File: USPT Oct 17, 2000

DOCUMENT-IDENTIFIER: US 6132729 A
TITLE: Combined tissue factor and chemotherapeutic methods and compositions for coagulation and tumor treatment

BSPR:
The antibody constructs and conjugates may be operatively attached to at least a first cytotoxic or otherwise anti-cellular agent. They may also be operatively attached to at least a first coagulation factor. In attachment to coagulants, bispecific constructs may also be advantageously employed (e.g., using two antibody binding regions), although the covalent linkages are generally preferred for use with the toxins. Any one or more of the toxic or coagulating agents known in the art may be employed in such "immunotoxins" or "coaguligands", and Tissue Factor or Tissue Factor derivatives may also be employed as part of the coaguligands, where the coaguligand is the second, "anti-cancer agent".

DRPR:
FIG. 12A and FIG. 12B: Inhibition of growth of C1300 Mu.gamma. tumors in mice by tTF.sub.219. FIG. 12A: Mice with 0.8 to 1.0 cm diameter C1300(Mu.gamma.) tumors were given two intravenous injections of B21-2/10H10-tTF coaguligand (.circle-solid.) spaced 6 days apart (arrows). Mice in control groups received equivalent doses of tTF alone (.quadrature.), CAMPATH-2/10H10 plus tTF (.DELTA.), or phosphate buffered saline (.largecircle.). Mice that received B21-2/OX7 and tTF had similar tumor responses to those in animals receiving tTF alone. Administration of B21-2/10H10 alone did not affect tumor growth. Each group contained 12 to 27 mice. Points represent the mean tumor volume per group (.+-.SEM). Mean

DEPR:
Despite the significant impairment of coagulative capacity of the tTF, tTF can promote blood coagulation when tethered or functionally associated by some other means with a phospholipid or membrane environment. For example, it is demonstrated herein that using a bispecific antibody that binds tTF to a plasma membrane antigen allows restoration of useful coagulating activity. This led one of the present inventors to develop methods for the specific coagulation of tumor vascular in vivo by using targeting constructs to deliver tTF or variants thereof specifically to the tumor vascular or stroma (WO 96/01653). Intravenous administration of such a "coaguligand" leads to localization of the coagulants within the tumor, thrombosis of the tumor vessels, and resultant tumor necrosis.

DEPR:
The development of the intelligent, targeted delivery of coagulants to the tumor vasculature, as exemplified using a bispecific targeting antibody-tTF composition, may be seen as representing an improvement over classic immunotoxin therapy. In fact, such coaguligand treatment induces thrombosis of tumor vessels in less than 30 minutes, in comparison to about 6 hours necessary to achieve the same effect following administration of an immunotoxin. Furthermore, there was no notable side effects as a result of the coaguligand treatment. Although the targeted delivery of a coagulant such as tTF was surprisingly effective, this stills requires the preparation of the "targeting construct".

DEPR:
The surprising finding of the inventors that tTF specifically localized within tumors sufficiently so as to cause anti-tumor effect was discovered during studies using tTF as a control in antibody-coagulant ("coaguligand") tumor targeting studies. From this initial discovery, the inventors developed the various aspects of the invention disclosed herein. The Tissue Factor compounds or constructs for use in the present invention have thus been developed from the original tTF first employed. Accordingly, various TF constructs may now be employed, including many different forms of tTF, longer but still impaired TFs, mutants TFs, any truncated, variant or mutant TFs modified or otherwise conjugated to improve their half-life, and all such functional equivalents thereof. However, it will be understood that each of the TF constructs for use in the invention are unified by the need to be "coagulation-deficient". As detailed herein below, there are various structural considerations that may be employed in the design of candidate coagulation-deficient TFs, and various assays are available for confirming that the candidate TFs are indeed suitable for use in the treatment aspects of the present invention. Given that the technological skills for creating a variety of compounds, e.g., using molecular biology, are routine to those of ordinary skill in the art, and given the extensive structural and functional guidance provided herein, the ordinary artisan will be readily able to make and use a number of different coagulation-deficient TFs in the context of the present invention.

DEPR:
In terms of surgery, any surgical intervention may be practiced in combination with the present invention. In connection with radiotherapy, any mechanism for inducing DNA damage locally within tumor cells is contemplated, such as .gamma.-irradiation, X-rays, UV-irradiation, microwaves and even electronic emissions and the like. The directed delivery of radioisotopes to tumor cells is also contemplated, and this may be used in connection with a targeting antibody or other targeting means. Cytokine therapy also has proven to be an effective partner for combined therapeutic regimens. Various cytokines may be employed in such combined approaches. Examples of cytokines include IL-1.alpha. IL-1.beta., IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, TGF-.beta., GM-CSF, M-CSF, G-CSF, TNF.alpha., TNF.beta., LAF, TCGF, BCGF, TRF, BAF, BDG, MP, LIF, OSM, TMF, PDGF, IFN-.alpha., IFN-.beta., IFN-.gamma.. Cytokines are administered according to standard regimens, consistent with clinical indications such as the condition of the patient and relative toxicity of the cytokine.

DEPR:
In certain embodiments, the present invention shows that the anti-tumor activity of tTF is enhanced when administered in combination with a chemotherapeutic agent. The mechanisms by which the drugs enhance the anti-tumor activity of tTF have not been precisely defined, but the inventors believe that the drug kills proliferating tumor cells creating necrotic areas that cause phagocytic cells to infiltrate the tumor. IL-1, TNF.alpha. and other cytokines released by the infiltrating cells then activate the tumor vascular endothelium making it better able to support coagulation by tTF, a generally weak thrombogen. The drug thus enhances the thrombotic action of tTF.

DEPR:
E2. Immunotoxin and Coaguligand Combinations and Therapy

DEPR:
Any one or more of the coagulation-deficient TF constructs of the invention may be used in combination with immunotoxins (ITs) and/or coaguligands in which the targeting portion thereof (e.g., antibody or ligand) is directed to a relatively specific marker of the tumor cells, tumor vasculature or tumor stroma. In common with the chemotherapeutic agents discussed above, it is possible that the use of a coagulation-deficient TF construct in combination with a targeted toxic agent (IT) or coagulant (coaguligand) will result in the distinct agents being directed against different targets within the tumor environment. This should result in additive, markedly greater than additive or even synergistic results.

DEPR:
With coaguligands, the burden of very stringent targeting, e.g., as imposed when using immunotoxins, is lessened. Therefore, to achieve specific targeting means that coagulation is promoted in the tumor vasculature relative to the vasculature in non-tumor sites. Thus, specific targeting of a coaguligand is a functional term rather than a purely physical term relating to the biodistribution properties of the targeting agent, and it is not unlikely that useful targets may be not be entirely tumor-restricted, and that targeting ligands which are effective to promote tumor-specific coagulation may nevertheless be found at other sites of the body following administration.

DEPR:
Another means of defining a tumor-associated target is in terms of the characteristics of the tumor cell, rather than describing the biochemical properties of an antigen expressed by the cell. Accordingly, the inventors contemplate that any antibody that preferentially binds to a tumor cell may be used as the targeting component of an immunotoxin or coaguligand. The preferential tumor cell binding is again based upon the antibody exhibiting high affinity for the tumor cell and not having significant reactivity with life-sustaining normal cells or tissues, as defined above.

DEPR:
The cells of the vasculature are intended as targets for use in the present invention. In these cases, at least one binding region of the immunotoxin or coaguligand will be capable of binding to an accessible marker preferentially expressed by disease-associated vasculature endothelial cells. The exploitation of the vascular markers is made possible due to the proximity of the vascular

endothelial cells to the disease area and to the products of the local aberrant physiological processes. For example, tumor vascular endothelial cells are exposed to tumor cells and tumor-derived products that change the phenotypic profile of the endothelial cells.

DEPR:
Binding of fibrinogen to its receptor alters the conformation of the carboxyl-terminal aspects of the A.alpha.-chains, exposing the sequences which reside in the coiled-coil connector segments between the D and E domains of the molecule, generating the RIBS epitopes. In practical terms, the RIBS sequences are proposed as epitopes for use in targeting with a coaguligand. The MAbs 9F9 and 155B16 may thus be advantageously used, as may the antibodies described by Zamarron et al. (1991).

DEPR:
p-155, a multimeric platelet protein that is expressed on activated platelets (Hayward et al., 1991), may be targeted using the invention. Platelets respond to a large number of stimuli by undergoing complex biochemical and morphological changes. These changes are involved in physiological processes including adhesion, aggregation, and coagulation. Platelet activation produces membrane alterations that can be recognized by monoclonal antibodies. The monoclonal antibody JS-1 (Hayward et al., 1991) is one such antibody contemplated for use as part of a coaguligand.

DEPR:
For certain applications, it is envisioned that the second therapeutic agents will be pharmacological agents attached to antibodies or growth factors, particularly cytotoxic or otherwise anti-cellular agents having the ability to kill or suppress the growth or cell division of endothelial cells. In general, the secondary aspects of the invention contemplate the use of any pharmacological agent that can be conjugated to a targeting agent, preferably an antibody, and delivered in active form to the targeted endothelium or stroma. Exemplary anti-cellular agents include chemotherapeutic agents, radioisotopes as well as cytotoxins. In the case of chemotherapeutic agents, the inventors propose that agents such as a hormone such as a steroid; an anti-metabolite such as cytosine arabinoside, fluorouracil, methotrexate or aminopterin; an anthracycline; mitomycin C; a vinca alkaloid; demecolcine; etoposide; mithramycin; or an anti-tumor alkylating agent such as chlorambucil or melphalan, will be particularly preferred. Other embodiments may include agents such as a cytokine, growth factor, bacterial endotoxin or the lipid A moiety of bacterial endotoxin. In any event, it is proposed that agents such as these may, if desired, be successfully conjugated to a targeting agent, preferably an antibody, in a manner that will allow their targeting, internalization, release or presentation to blood components at the site of the targeted endothelial cells as required using known conjugation technology (see, e.g., Ghose et al., 1983 and Ghose et al., 1987).

DEPR:
It is contemplated that patients chosen for combined studies would have failed to respond to at least one course of conventional therapy and had to have objectively measurable disease as determined by physical examination, laboratory techniques, or radiographic procedures. Where murine monoclonal antibody portions are employed in the immunotoxins or coaguligands, the patients should have no history of allergy to mouse immunoglobulin. Any chemotherapy should be stopped at least 2 weeks before entry into the study.

DEPR:
These Tissue Factor and IT or coaguligand combinations may be administered over a period of approximately 4-24 hours, with each patient receiving 2-4 infusions at 2-7 day intervals. Administration can also bc performed by a steady rate of infusion over a 7 day period. The infusion given at any dose level should be dependent upon any toxicity observed. Hence, if Grade II toxicity was reached after any single infusion, or at a particular period of time for a steady rate infusion, further doses should be withheld or the steady rate infusion stopped unless toxicity improved. Increasing doses of Tissue Factor with either immunotoxins or coaguligands should be administered to groups of patients until approximately 60% of patients showed unacceptable Grade III or IV toxicity in any category. Doses that are 2/3 of this value could be defined as the safe dose.

DEPR:
Physical examination, tumor measurements, and laboratory tests should, of course, be performed before treatment and at intervals up to 1 month later. Laboratory tests should include complete blood counts, serum creatinine, creatine kinase, electrolytes, urea, nitrogen, SGOT, bilirubin, albumin, and total serum protein. Serum samples taken up to 60 days after treatment should be evaluated by radioimmunoassay for the presence of the intact Tissue Factor, immunotoxin and/or coaguligand or components thereof and antibodies against any portions thereof. Immunological analyses of sera, using any standard assay such as, for example, an ELISA or RIA, will allow the pharmacokinetics and clearance of the therapeutic agent to be evaluated.

DEPR:
This culturing provides a population of hybridomas from which specific hybridomas are selected. Typically, selection of hybridomas is performed by culturing the cells by single-clone dilution in microtiter plates, followed by testing the individual clonal supernatants (after about two to three weeks) for the desired reactivity. The assay should be sensitive, simple and rapid, such as radioimmunoassays, enzyme immunoassays, cytotoxicity assays, plaque assays, dot immunobinding assays, and the like.

DEPR:
A histological study was performed to determine whether intravenous administration of the B21-2/10H10-tTF coaguligand induced selective thrombosis of tumor vasculature in mice bearing subcutaneous C1300(Mu.gamma.) neuroblastomas of 0.8 to 1.0 cm diameter (FIG. 9). Within 30 minutes, all vessels throughout the tumor were thrombosed, containing occlusive platelet aggregates, packed erythrocytes, and fibrin. At this time, tumor cells were indistinguishable histologically from tumor cells of untreated mice.

DEPR:
After 4 hours, however, there were signs of tumor cell injury. The majority of tumor cells had separated from one another and had pyknotic nuclei, and the tumor interstitium commonly contained erythrocytes. By 24 hours, the tumor showed advanced necrosis, and by 72 hours, the entire central region of the tumor had condensed into amorphous debris. These studies indicated that the predominant occlusive effect of the B21-2/10H10-tTF coaguligand on tumor vessels is mediated through binding to Class II antigens on tumor vascular endothelium.

DEPR:
Coaguligand treatment was well tolerated, mice lost no weight and retained normal appearance and activity levels. At the treatment dose of 0.6 mg/kg B21-2/10H10 plus 0.5 mg/kg tTF, toxicity was observed in only two of forty mice (thrombosis of tail vein). It is important to note that neither thrombi, nor histological or morphological abnormalities were visible in paraffin sections of liver, kidney, lung, intestine, heart, brain, adrenals, pancreas, or spleen from the tumor-bearing mice 30 minutes or 24 hours after administration of coaguligand or free tTF. Furthermore, no signs of toxicity (behavioral changes, physical signs, weight changes) were observed in treated animals.

DEPR:
The inventors next investigated whether intravenous administration of the B21-2/10H10-tTF coaguligand could inhibit the growth of large (0.8 to 1.0 cm diameter) tumors in mice. The pooled results from three separate studies indicate that mice receiving B21-2/10H10-tTF coaguligand had complete tumor regressions lasting four months or more. These anti-tumor effects were significantly greater than for all other treatment groups (FIG. 12A).

DEPR:
Surprisingly, the inventors found that the anti-tumor effect of the B21-2/10H10-tTF coaguligand was attributable, in part, to a non-targeted effect of tTF. Tumors in mice receiving tTF alone or mixed with control

DEPR:
In animals that did not show complete tumor regression after B21-2/10H10-tTF coaguligand treatment, the tumors grew back from a surviving microscopic rim of cells at the periphery of the tumor. Immunohistochemical examination of these tumors revealed that the vascular endothelium at the invading edge of the tumors lacked detectable Class II antigens, consistent with a lack of thrombosis of

these vessels by the coaguligand permitting local tumor cell survival. Thus, coadministration of a drug acting on the tumor cells themselves would likely improve efficacy, as has been observed with another antivascular therapy (Burrows and Thorpe, 1992;Burrows and Thorpe 1993;Burrows and Thorpe 1994;U.S. Ser. Nos. 07/846,349; 08/205,330; 08/295,868;and 08/350,212).

DEPR:
The inventors previously demonstrated that a powerfully cytotoxic ricin A-chain immunotoxin directed against the tumor cells themselves was virtually devoid of anti-tumor activity when administered to mice with large C1300(Mu.gamma.) tumors (Burrows and Thorpe, 1993; U.S. Ser. Nos. 07/846,349; 08/205,330; 08/295,868;and 08/350,212). The lack of activity was due to the inability of the immunotoxin to gain access to tumor cells in large tumor masses, thus attesting to the comparative effectiveness of coaguligand therapy.

DEPR:
The studies using coaguligands confirm the therapeutic potential of selective initiation of the blood coagulation cascade in tumor vasculature (U.S. Ser. Nos. 08/273,567; 08/482,369; 08/485,482; 08/487,427; 08/479,733; 08/472,631; 08/479,727;and 08/481,904). The induction of tumor infarction by targeting a thrombogen to tumor endothelial cell markers is therefore an effective anti-cancer strategy and may even result in the eradication of primary solid tumors and vascularized metastases.

DEPU:
Wawrzynczak and Thorpe In: Immunoconjugates: Antibody Conjugates in Radioimaging and Therapy of Cancer, Vogel (ed.), New York, Oxford University Press, pp. 28-55, 1987.

☐ 3. Document ID: US 6093399 A

L5: Entry 3 of 7 File: USPT Jul 25, 2000

DOCUMENT-IDENTIFIER: US 6093399 A
TITLE: Methods and compositions for the specific coagulation of vasculature

DRPR:
FIG. 3A, FIG. 3B, FIG. 3C and FIG. 3D. Time course of vascular thrombosis and tumor necrosis after administration of coaguligand. Groups of 3 mice bearing 0.8 cm diameter C1300 (Mu.gamma.) tumors were given an intravenous injection of a coaguligand composed of 14 .mu.g B21-2/10H10 and 11 .mu.g tTF. FIG. 3A; Before injection: blood vessels are intact and tumor cells are healthy. FIG. 3B; 0.5 hours: blood vessels throughout the tumor are thrombosed; tumor cells are healthy. FIG. 3C; 4 hours: dense thrombi are present in all tumor vessels and tumor cells are separating and developing pyknotic nuclei. Erythrocytes are visible in the tumor interstitium. FIG. 3D; 24 hours: advanced tumor necrosis throughout the tumor. Arrows indicate blood vessels.

DRPR:
FIG. 4. Solid tumor regression induced by tumor-vasculature directed coaguligand therapy. Nu/nu mice bearing approximately 0.8 cm diameter C1300 (Mu.gamma.) tumors were given two intravenous injections of B21-2/10H10 (14 .mu.g) mixed with tTF (11 .mu.g) spaced 1 week apart (arrows) (.quadrature.). Mice in control groups received equivalent doses of tTF alone (.circle-solid.), B21-2/10H10 alone (.largecircle.) or diluent (.box-solid.). Other control groups which received equivalent doses of isotype-matched control bispecific antibodies (SFR8/10H10, OX7/10H10 or B21-2/OX7) and tTF had similar tumor responses to those in animals receiving tTF alone. The number of mice per group was 7 or 8.

DEPR:
To conduct an antibody competition study between TEC-4 or TEC-11 and any test

antibody, one may first label TEC-4 or TEC-11 with a detectable label, such as, e.g., biotin or an enzymatic or radioactive label, to enable subsequent identification. In these cases, one would incubate the labelled antibodies with the test antibodies to be examined at various ratios (e.g., 1:1, 1:10 and 1:100) and, after a suitable period of time, one would then assay the reactivity of the labelled TEC-4 or TEC-11 antibodies and compare this with a control value in which no potentially competing antibody (test) was included in the incubation.

DEPR:
The assay may be any one of a range of immunological assays based upon antibody binding and the TEC-4 or TEC-11 antibodies would be detected by means of detecting their label, e.g., using streptavidin in the case of biotinylated antibodies or by using a chromogenic substrate in connection with an enzymatic label or by simply detecting the radiolabel. An antibody that binds to the same epitope as TEC-4 or TEC-11 will be able to effectively compete for binding and thus will significantly reduce TEC-4 or TEC-11 binding, as evidenced by a reduction in labelled antibody binding. In the present case, after mixing the labelled TEC-4 or TEC-11 antibodies with the test antibodies, suitable assays to determine the remaining reactivity include, e.g., ELISAs, RIAs or western blots using human endoglin; immunoprecipitation of endoglin; ELISAs, RIAs or immunofluorescent staining of recombinant cells expressing human endoglin; indirect immunofluorescent staining of tumor vasculature endothelial cells; reactivity with HUVEC or TCM-activated HUVEC cell surface determinants indirect immunofluorescence and FACS analysis. This latter method is most preferred and was employed to show that the epitopes recognized by TEC-4 and TEC-11 are distinct from that of 44G4 (Gougos & Letarte, 1988).

DEPR:
Binding of fibrinogen to its receptor alters the conformation of the carboxyl-terminal aspects of the A.alpha.-chains, exposing the sequences which reside in the coiled-coil connector segments between the D and E domains of the molecule, generating the RIBS epitopes. In practical terms, the RIBS sequences are proposed as epitopes for use in targeting with a coaguligand. The MAbs 9F9 and 155B16 may thus be advantageously used, as may the antibodies described by Zamarron et al. (1991).

DEPR:
biochemical and morphological changes. These changes are involved in physiological processes including adhesion, aggregation, and coagulation. Platelet activation produces membrane alterations that can be recognized by monoclonal antibodies. The monoclonal antibody JS-1 (Hayward et al., 1991) is one such antibody contemplated for use as part of a coaguligand.

DEPR:
To assess the targeting region binding function, all that is required is to conduct a binding assay to ensure that the bispecific ligand still binds to the targeted component in substantially the same manner as the uncomplexed first binding region. The suitable binding assays are of the type usually seen in immunological binding assays, where the first targeting region is an antibody, and/or other biochemical binding assays, e.g., those using .sup.125 Iodine labeled proteins or other radiolabeled components, as used to assess ligand-receptor binding, to generate Scatchard plots, and the like.

DEPR:
Where the bispecific ligand includes a second binding region that binds to a coagulant, e.g.., it is a bispecific antibody, further useful assays are those of the type that allow the binding functions of both arms of the bispecific ligand to be assessed at the same time. For example, this may be achieved by assessing the binding of a radiolabeled coagulant to a target cell via bridging with the bispecific ligand or antibody. Such an assay is exemplified by the binding of tTF to target cells using the B21-2/10H10 bispecific antibody, as described in Example II.

DEPR:
This culturing provides a population of hybridomas from which specific hybridomas are selected. Typically, selection of hybridomas is performed by culturing the cells by single-clone dilution in microtiter plates, followed by testing the individual clonal supernatants (after about two to three weeks) for the desired reactivity. The assay should be sensitive, simple and rapid, such as

radioimmunoassays, enzyme immunoassays, cytotoxicity assays, plaque assays, dot immunobinding assays, and the like.

DEPR:
The present inventors herein demonstrate that, in a C1300 (Mu.gamma.) tumor bearing mouse, the anti-MHC Class II/anti-TF bispecific antibody is able to induce coagulation specifically in the vasculature of the tumor when administered together with tTF. Indeed, intravenous administration of the antibody:tTF complex induced rapid thrombosis of tumor vasculature and complete tumor regressions in 70% of animals. Neither the bispecific antibody alone, nor tTF alone, nor any of the isotype matched control antibodies in the presence or absence of tTF, was able to elicit the same effect. This indicates that the B21-2/10H10 bispecific antibody acts as a "coaguligand" that is capable of bridging target cells and tTF so that tTF can activate factor X and start the coagulation cascade. It also shows the evident success of the coaguligand in treating solid tumors.

DEPR:
The present example describes the synthesis of a bispecific antibody capable of specifically directing a coagulant to a tumor site, i.e., a "coaguligand".

DEPR:
The present example shows the bispecificity of the coagulating antibody (coaguligand) and demonstrates that specific binding, cellular delivery and coagulation is achieved in vitro using the coaguligand.

DEPR:
3. Radiolabeling of Proteins

DEPR:
25 .mu.l of .sup.125 I-tTF at 8 .mu.g/ml in the same buffer were added to each well, giving a molar excess of tTF. The plates were shaken and incubated for 1 hr at 4.degree. C. The cells were then washed 3.times. in the plates with 0.9% (w/v) NaCl containing 2 mg/ml BSA. The contents of the wells were pipetted over a 10:11 (v/v) mixture of dibutyl phthalate and bis(2-ethylhexyl)phthalate oils in microcentrifuge tubes. The tubes were centrifuged for 1.5 min at 7500 g and were snap frozen in liquid nitrogen. The tips containing the cells were cut off. The radioactivity in the cell pellet and in the supernatant was measured in a gamma counter.

DEPR:
The present example describes the specific coagulation of tumor vasculature in vivo that results following the administration of the bispecific antibody coaguligand as a delivery vehicle for human tissue factor.

DEPR:
Intravenous administration of a coaguligand composed of B21-2/10H10 (20 .mu.g) and tTF (16 .mu.g) to mice bearing solid C1300 (Mu.gamma.) tumors caused tumors to assume a blackened, bruised appearance within 30 minutes. A histological study of the time course of events within the tumor revealed that 30 minutes after injection of coaguligand all vessels in all regions of the tumor were thrombosed (FIG. 3B). Vessels contained platelet aggregates, packed red cells and fibrin. At this time, tumor-cells were healthy, being indistinguishable morphologically from tumor cells in untreated mice (FIG. 3A).

DEPR:
No thrombi or morphological abnormalities were visible in paraffin sections of liver, kidney, lung, intestine, heart, brain, adrenals, pancreas and spleen taken from tumor-bearing mice 30 minutes, 4 hours and 24 hours after administration of coaguligand or tTF.

DEPR:
FIG. 4 shows the results of a representative anti-tumor study in which a coaguligand composed of B21-2/10H10 and tTF was administered to mice with 0.8 cm diameter tumors. The tumors regressed to approximately half their pretreatment size. Repeating the treatment on the 7th day caused the tumors to regress further, usually completely. In 5/7 animals, complete regressions were obtained. Two of the mice subsequently relapsed four and six months later. These anti-tumor effects are statistically highly significant ($P<0.001$) when compared with all other groups.

DEPR:
Tumors in mice treated with tTF alone or with tTF mixed with the isotype-matched control bispecific antibodies, SFR8/10H10 or B21-2/OX7, grew more slowly than those in groups receiving antibodies or diluent alone. These differences were statistically significant ($P<0.05$) on days 12-14. Thus, part of the anti-tumor effects seen with the B21-2/10H10 coaguligand are attributable to a slight non-specific action of tTF itself.

DEPR:
At the end of the study, two mice which had been treated with diluent alone and which had very large tumors of 2.0 cm.sup.3 and 2.7 cm.sup.3 (i.e. 10-15% of their body weight) were given coaguligand therapy. Both had complete remissions although their tumors later regrew at the original site of tumor growth.

DEPR:
The present studies show that soluble human tTF, possessing practically no ability to induce coagulation, became a powerful thrombogen for tumor vasculature when targeted by means of a bispecific antibody to tumor endothelial cells. In vitro coagulation studies showed that the restoration of thrombotic activity of tTF is mediated through its cross-linking to antigens on the cell surface.

DEPR:
Administration of a coaguligand directed against class II to mice having tumors with class II-expressing vasculature caused rapid thrombosis of blood vessels throughout the tumor. This was followed by infarction of the tumor and complete tumor regressions in a majority of animals. In those animals where complete regressions were not obtained, the tumors grew back from a surviving rim of tumor cells on the periphery of the tumor where it had infiltrated into the surrounding normal tissues. The vessels at the growing edge of the tumor lacked class II antigens, thus explaining the lack of thrombosis of these vessels by the coaguligand. It is likely that these surviving cells would have been killed by coadministering a drug acting on the tumor cells themselves, as was found previously (Burrows & Thorpe, 1993).

DEPR:
The anti-tumor effects of the coaguligand were similar in magnitude to those obtained in the same tumor model with an immunotoxin composed of anti-class II antibody and deglycosylated ricin A-chain (Burrows & Thorpe, 1993). One difference between the two agents is their rapidity of action. The coaguligand induced thrombosis of tumor vessels in less than 30 minutes whereas the immunotoxin took 6 hours to achieve the same effect. The immunotoxin acts more slowly because thrombosis is secondary to endothelial cell damage caused by the shutting down of protein syntheses.

DEPR:
A second and important difference between the immunotoxin and the coaguligand is that they have different toxic side effects. The immunotoxin caused a lethal destruction of class II-expressing gastrointestinal epithelium unless antibiotics were given to suppress class II induction by intestinal bacteria. The coaguligand caused no gastrointestinal damage, as expected because of the absence of clotting factors outside of the blood, but caused coagulopathies in occasional mice when administered at high dosage.

DEPR:
It is contemplated that patients chosen for the study would have failed to respond to at least one course of conventional therapy and had to have objectively measurable disease as determined by physical examination, laboratory techniques, or radiographic procedures. Where murine monoclonal antibody portions are employed, the patients should have no history of allergy to mouse immunoglobulin. Any chemotherapy should be stopped at least 2 weeks before entry into the study.

DEPR:
Physical examination, tumor measurements, and laboratory tests should, of course, be performed before treatment and at intervals up to 1 month later. Laboratory tests should include complete blood counts, serum creatinine, creatine kinase, electrolytes, urea, nitrogen, SGOT, bilirubin, albumin, and total serum protein. Serum samples taken up to 60 days after treatment should be evaluated by

radioimmunoassay for the presence of the intact bispecific ligand or components thereof and antibodies against any or both portions of the ligand. Immunological analyses of sera, using any standard assay such as, for example, an ELISA or RIA, will allow the pharmacokinetics and clearance of the therapeutic agent to be evaluated.

DEPU:
Griffin et al., Proc. 2nd Conf. on Radioimmunodetection & Therapy of Cancer, 82, 1988a.

DEPU:
Murray et al., Radio. Onc., 16:221-234, 1989.

DEPU:
Wawrzynczak & Thorpe, "Methods for preparing immunotoxins: effect of the linkage on activity and stability", in: Immunoconjugates,: Antibody conjugates in radioimaging and therapy of cancer, Vogel (ed), New York, Oxfod University Press, pp. 28-55, 1987.

ORPL:
Epenetos et al., "Limitations of Radiolabeled Monoclonal Antibodies for Localization of Human Neoplasms," Cancer Res., 46:3183-3191, 1986.

☐ 4. Document ID: US 6036955 A

L5: Entry 4 of 7 File: USPT Mar 14, 2000

DOCUMENT-IDENTIFIER: US 6036955 A
TITLE: Kits and methods for the specific coagulation of vasculature

DRPR:
FIG. 3A, FIG. 3B, FIG. 3C and FIG. 3D. Time course of vascular thrombosis and tumor necrosis after administration of coaguligand. Groups of 3 mice bearing 0.8 cm diameter C1300 (Mu.gamma.) tumors were given an intravenous injection of a coaguligand composed of 14 .mu.g B21-2/10H10 and 11 .mu.g tTF. FIG. 3A; Before injection: blood vessels are intact and tumor cells are healthy. FIG. 3B; 0.5 hours: blood vessels throughout the tumor are thrombosed; tumor cells are healthy. FIG. 3C; 4 hours: dense thrombi are present in all tumor vessels and tumor cells are separating and developing pyknotic nuclei. Erythrocytes are visible in the tumor interstitium. FIG. 3D; 24 hours: advanced tumor necrosis throughout the tumor. Arrows indicate blood vessels.

DRPR:
FIG. 4. Solid tumor regression induced by tumor-vasculature directed coaguligand therapy. Nu/nu mice bearing approximately 0.8 cm diameter C1300 (Mu.gamma.) tumors were given two intravenous injections of B21-2/10H10 (14 .mu.g) mixed with tTF (11 .mu.g) spaced 1 week apart (arrows) (.quadrature.). Mice in control groups received equivalent doses of tTF alone (.circle-solid.), B21-2/10H10 alone (O) or diluent (.box-solid.). Other control groups which received equivalent doses of isotype-matched control bispecific antibodies (SFR8/10H10, OX7/10H10 or B21-2/OX7) and tTF had similar tumor responses to those in animals receiving tTF alone. The number of mice per group was 7 or 8.

DEPR:
To conduct an antibody competition study between TEC-4 or TEC-11 and any test antibody, one may first label TEC-4 or TEC-11 with a detectable label, such as, e.g., biotin or an enzymatic or radioactive label, to enable subsequent identification. In these cases, one would incubate the labelled antibodies with the test antibodies to be examined at various ratios (e.g., 1:1, 1:10 and 1:100) and, after a suitable period of time, one would then assay the reactivity of the labelled TEC-4 or TEC-11 antibodies and compare this with a control value in

which no potentially competing antibody (test) was included in the incubation.

DEPR:
The assay may be any one of a range of immunological assays based upon antibody binding and the TEC-4 or TEC-11 antibodies would be detected by means of detecting their label, e.g., using streptavidin in the case of biotinylated antibodies or by using a chromogenic substrate in connection with an enzymatic label or by simply detecting the radiolabel. An antibody that binds to the same epitope as TEC-4 or TEC-11 will be able to effectively compete for binding and thus will significantly reduce TEC-4 or TEC-11 binding, as evidenced by a reduction in labelled antibody binding. In the present case, after mixing the labelled TEC-4 or TEC-11 antibodies with the test antibodies, suitable assays to determine the remaining reactivity include, e.g., ELISAs, RIAs or western blots using human endoglin; immunoprecipitation of endoglin; ELISAs, RIAs or immunofluorescent staining of recombinant cells expressing human endoglin; indirect immunofluorescent staining of tumor vasculature endothelial cells; reactivity with HUVEC or TCM-activated HUVEC cell surface determinants indirect immunofluorescence and FACS analysis. This latter method is most preferred and was employed to show that the epitopes recognized by TEC-4 and TEC-11 are distinct from that of 44G4 (Gougos & Letarte, 1988).

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Binding of fibrinogen to its receptor alters the conformation of the carboxyl-terminal aspects of the A.alpha.-chains, exposing the sequences which reside in the coiled-coil connector segments between the D and E domains of the molecule, generating the RIBS epitopes. In practical terms, the RIBS sequences are proposed as epitopes for use in targeting with a coaguligand. The MAbs 9F9 and 155B16 may thus be advantageously used, as may the antibodies described by Zamarron et al. (1991).

DEPR:
p-155, a multimeric platelet protein that is expressed on activated platelets (Hayward et al., 1991), may be targeted using the invention. Platelets respond to a large number of stimuli by undergoing complex biochemical and morphological changes. These changes are involved in physiological processes including adhesion, aggregation, and coagulation. Platelet activation produces membrane alterations that can be recognized by monoclonal antibodies. The monoclonal antibody JS-1 (Hayward et al., 1991) is one such antibody contemplated for use as part of a coaguligand.

DEPR:
To assess the targeting region binding function, all that is required is to conduct a binding assay to ensure that the bispecific ligand still binds to the targeted component in substantially the same manner as the uncomplexed first binding region. The suitable binding assays are of the type usually seen in immunological binding assays, where the first targeting region is an antibody, and/or other biochemical binding assays, e.g., those using .sup.125 Iodine labeled proteins or other radiolabeled components, as used to assess ligand-receptor binding, to generate Scatchard plots, and the like.

DEPR:
Where the bispecific ligand includes a second binding region that binds to a coagulant, e.g., it is a bispecific antibody, further useful assays are those of the type that allow the binding functions of both arms of the bispecific ligand to be assessed at the same time. For example, this may be achieved by assessing the binding of a radiolabeled coagulant to a target cell via bridging with the bispecific ligand or antibody. Such an assay is exemplified by the binding of tTF to target cells using the B21-2/10H10 bispecific antibody, as described in Example II.

DEPR:
This culturing provides a population of hybridomas from which specific hybridomas are selected. Typically, selection of hybridomas is performed by culturing the cells by single-clone dilution in microtiter plates, followed by testing the individual clonal supernatants (after about two to three weeks) for the desired reactivity. The assay should be sensitive, simple and rapid, such as radioimmunoassays, enzyme immunoassays, cytotoxicity assays, plaque assays, dot immunobinding assays, and the like.

DEPR:
The present inventors herein demonstrate that, in a C1300 (Mu.gamma.) tumor bearing mouse, the anti-MHC Class II/anti-TF bispecific antibody is able to induce coagulation specifically in the vasculature of the tumor when administered together with tTF. Indeed, intravenous administration of the antibody:tTF complex induced rapid thrombosis of tumor vasculature and complete tumor regressions in 70% of animals. Neither the bispecific antibody alone, nor tTF alone, nor any of the isotype matched control antibodies in the presence or absence of tTF, was able to elicit the same effect. This indicates that the B21-2/10H10 bispecific antibody acts as a "coaguligand" that is capable of bridging target cells and tTF so that tTF can activate factor X and start the coagulation cascade. It also shows the evident success of the coaguligand in treating solid tumors.

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The present example describes the synthesis of a bispecific antibody capable of specifically directing a coagulant to a tumor site, i.e., a "coaguligand".

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The present example shows the bispecificity of the coagulating antibody (coaguligand) and demonstrates that specific binding, cellular delivery and coagulation is achieved in vitro using the coaguligand.

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25 .mu.l of .sup.125 I-tTF at 8 .mu.g/ml in the same buffer were added to each well, giving a molar excess of tTF. The plates were shaken and incubated for 1 hr at 4.degree. C. The cells were then washed 3.times. in the plates with 0.9% (w/v) NaCl containing 2 mg/ml BSA. The contents of the wells were pipetted over a 10:11 (v/v) mixture of dibutyl phthalate and bis(2-ethylhexyl)phthalate oils in microcentrifuge tubes. The tubes were centrifuged for 1.5 min at 7500 g and were snap frozen in liquid nitrogen. The tips containing the cells were cut off. The radioactivity in the cell pellet and in the supernatant was measured in a gamma counter.

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No thrombi or morphological abnormalities were visible in paraffin sections of liver, kidney, lung, intestine, heart, brain, adrenals, pancreas and spleen taken from tumor-bearing mice 30 minutes, 4 hours and 24 hours after administration of coaguligand or tTF.

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FIG. 4 shows the results of a representative anti-tumor study in which a coaguligand composed of B21-2/10H10 and tTF was administered to mice with 0.8 cm diameter tumors. The tumors regressed to approximately half their pretreatment size. Repeating the treatment on the 7th day caused the tumors to regress further, usually completely. In 5/7 animals, complete regressions were obtained. Two of the mice subsequently relapsed four and six months later. These anti-tumor effects are statistically highly significant ($P<0.001$) when compared with all other groups.

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Tumors in mice treated with tTF alone or with tTF mixed with the isotype-matched control bispecific antibodies, SFR8/10H10 or B21-2/OX7, grew more slowly than those in groups receiving antibodies or diluent alone. These differences were statistically significant ($P<0.05$) on days 12-14. Thus, part of the anti-tumor

effects seen with the B21-2/10H10 coaguligand are attributable to a slight non-specific action of tTF itself.

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At the end of the study, two mice which had been treated with diluent alone and which had very large tumors of 2.0 cm.sup.3 and 2.7 cm.sup.3 (i.e. 10-15% of their body weight) were given coaguligand therapy. Both had complete remissions although their tumors later regrew at the original site of tumor growth.

DEPR:
The present studies show that soluble human tTF, possessing practically no ability to induce coagulation, became a powerful thrombogen for tumor vasculature when targeted by means of a bispecific antibody to tumor endothelial cells. In vitro coagulation studies showed that the restoration of thrombotic activity of tTF is mediated through its cross-linking to antigens on the cell surface.

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Administration of a coaguligand directed against class II to mice having tumors with class II-expressing vasculature caused rapid thrombosis of blood vessels throughout the tumor. This was followed by infarction of the tumor and complete tumor regressions in a majority of animals. In those animals where complete regressions were not obtained, the tumors grew back from a surviving rim of tumor cells on the periphery of the tumor where it had infiltrated into the surrounding normal tissues. The vessels at the growing edge of the tumor lacked class II antigens, thus explaining the lack of thrombosis of these vessels by the coaguligand. It is likely that these surviving cells would have been killed by coadministering a drug acting on the tumor cells themselves, as was found previously (Burrows & Thorpe, 1993).

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The anti-tumor effects of the coaguligand were similar in magnitude to those obtained in the same tumor model with an immunotoxin composed of anti-class II antibody and deglycosylated ricin A-chain (Burrows & Thorpe, 1993). One difference between the two agents is their rapidity of action. The coaguligand induced thrombosis of tumor vessels in less than 30 minutes whereas the immunotoxin took 6 hours to achieve the same effect. The immunotoxin acts more slowly because thrombosis is secondary to endothelial cell damage caused by the shutting down of protein syntheses.

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A second and important difference between the immunotoxin and the coaguligand is that they have different toxic side effects. The immunotoxin caused a lethal destruction of class II-expressing gastrointestinal epithelium unless antibiotics were given to suppress class II induction by intestinal bacteria. The coaguligand caused no gastrointestinal damage, as expected because of the absence of clotting factors outside of the blood, but caused coagulopathies in occasional mice when administered at high dosage.

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It is contemplated that patients chosen for the study would have failed to respond to at least one course of conventional therapy and had to have objectively measurable disease as determined by physical examination, laboratory techniques, or radiographic procedures. Where murine monoclonal antibody portions are employed, the patients should have no history of allergy to mouse immunoglobulin. Any chemotherapy should be stopped at least 2 weeks before entry into the study.

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Physical examination, tumor measurements, and laboratory tests should, of course, be performed before treatment and at intervals up to 1 month later. Laboratory tests should include complete blood counts, serum creatinine, creatine kinase, electrolytes, urea, nitrogen, SGOT, bilirubin, albumin, and total serum protein. Serum samples taken up to 60 days after treatment should be evaluated by radioimmunoassay for the presence of the intact bispecific ligand or components thereof and antibodies against any or both portions of the ligand. Immunological analyses of sera, using any standard assay such as, for example, an ELISA or RIA, will allow the pharmacokinetics and clearance of the therapeutic agent to be evaluated.

DEPU:
3. Radiolabeling of Proteins

DEPU:
Griffin et al., Proc. 2nd Conf. on Radioimmunodetection & Therapy of Cancer, 82, 1988a.

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Murray et al., Radio. Onc., 16:221-234, 1989.

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Wawrzynczak & Thorpe, "Methods for preparing immunotoxins: effect of the linkage on activity and stability", in: Immunoconjugates,: Antibody conjugates in radioimaging and therapy of cancer, Vogel (ed), New York, Oxfod University Press, pp. 28-55, 1987.

ORPL:
Epenetos et al., "Limitations of Radiolabeled Monoclonal Antibodies for Localization of Human Neoplasms," Cancer Res., 46:3183-3191, 1986.

☐ 5. Document ID: US 6004555 A

L5: Entry 5 of 7 File: USPT Dec 21, 1999

DOCUMENT-IDENTIFIER: US 6004555 A
TITLE: Methods for the specific coagulation of vasculature

DRPR:
FIG. 3A, FIG. 3B, FIG. 3C and FIG. 3D. Time course of vascular thrombosis and tumor necrosis after administration of coaguligand. Groups of 3 mice bearing 0.8 cm diameter C1300 (Mu.gamma.) tumors were given an intravenous injection of a coaguligand composed of 14 .mu.g B21-2/10H10 and 11 .mu.g tTF. FIG. 3A; Before injection: blood vessels are intact and tumor cells are healthy. FIG. 3B; 0.5 hours: blood vessels throughout the tumor are thrombosed; tumor cells are healthy. FIG. 3C; 4 hours: dense thrombi are present in all tumor vessels and tumor cells are separating and developing pyknotic nuclei. Erythrocytes are visible in the tumor interstitium. FIG. 3D; 24 hours: advanced tumor necrosis throughout the tumor. Arrows indicate blood vessels.

DRPR:
FIG. 4. Solid tumor regression induced by tumor-vasculature directed coaguligand therapy. Nu/nu mice bearing approximately 0.8 cm diameter C1300 (Mu.gamma.) tumors were given two intravenous injections of B21-2/10H10 (14 .mu.g) mixed with tTF (11 .mu.g) spaced 1 week apart (arrows) (.quadrature.). Mice in control groups received equivalent doses of tTF alone (.circle-solid.), B21-2/10H10 alone (.smallcircle.) or diluent (.box-solid.). Other control groups which received equivalent doses of isotype-matched control bispecific antibodies (SFR8/10H10, OX7/10H10 or B21-2/OX7) and tTF had similar tumor responses to those in animals receiving tTF alone. The number of mice per group was 7 or 8.

DRPR:
To conduct an antibody competition study between TEC-4 or TEC-11 and any test antibody, one may first label TEC-4 or TEC-11 with a detectable label, such as, e.g., biotin or an enzymatic or radioactive label, to enable subsequent identification. In these cases, one would incubate the labelled antibodies with the test antibodies to be examined at various ratios (e.g., 1:1, 1:10 and 1:100) and, after a suitable period of time, one would then assay the reactivity of the labelled TEC-4 or TEC-11 antibodies and compare this with a control value in which no potentially competing antibody (test) was included in the incubation.

DRPR:

The assay may be any one of a range of immunological assays based upon antibody binding and the TEC-4 or TEC-11 antibodies would be detected by means of detecting their label, e.g., using streptavidin in the case of biotinylated antibodies or by using a chromogenic substrate in connection with an enzymatic label or by simply detecting the radiolabel. An antibody that binds to the same epitope as TEC-4 or TEC-11 will be able to effectively compete for binding and thus will significantly reduce TEC-4 or TEC-11 binding, as evidenced by a reduction in labelled antibody binding. In the present case, after mixing the labelled TEC-4 or TEC-11 antibodies with the test antibodies, suitable assays to determine the remaining reactivity include, e.g., ELISAs, RIAs or western blots using human endoglin; immunoprecipitation of endoglin; ELISAs, RIAs or immunofluorescent staining of recombinant cells expressing human endoglin; indirect immunofluorescent staining of tumor vasculature endothelial cells; reactivity with HUVEC or TCM-activated HUVEC cell surface determinants indirect immunofluorescence and FACS analysis. This latter method is most preferred and was employed to show that the epitopes recognized by TEC-4 and TEC-11 are distinct from that of 44G4 (Gougos & Letarte, 1988).

DRPR:
Binding of fibrinogen to its receptor alters the conformation of the carboxyl-terminal aspects of the A.alpha.-chains, exposing the sequences which reside in the coiled-coil connector segments between the D and E domains of the molecule, generating the RIBS epitopes. In practical terms, the RIBS sequences are proposed as epitopes for use in targeting with a coaguligand. The MAbs 9F9 and 155B16 may thus be advantageously used, as may the antibodies described by Zamarron et al. (1991).

DRPR:
p-155, a multimeric platelet protein that is expressed on activated platelets (Hayward et al., 1991), may be targeted using the invention. Platelets respond to a large number of stimuli by undergoing complex biochemical and morphological changes. These changes are involved in physiological processes including adhesion, aggregation, and coagulation. Platelet activation produces membrane alterations that can be recognized by monoclonal antibodies. The monoclonal antibody JS-1 (Hayward et al., 1991) is one such antibody contemplated for use as part of a coaguligand.

DRPR:
To assess the targeting region binding function, all that is required is to conduct a binding assay to ensure that the bispecific ligand still binds to the targeted component in substantially the same manner as the uncomplexed first binding region. The suitable binding assays are of the type usually seen in immunological binding assays, where the first targeting region is an antibody, and/or other biochemical binding assays, e.g., those using .sup.125 Iodine labeled proteins or other radiolabeled components, as used to assess ligand-receptor binding, to generate Scatchard plots, and the like.

DRPR:
Where the bispecific ligand includes a second binding region that binds to a coagulant, e.g., it is a bispecific antibody, further useful assays are those of the type that allow the binding functions of both arms of the bispecific ligand to be assessed at the same time. For example, this may be achieved by assessing the binding of a radiolabeled coagulant to a target cell via bridging with the bispecific ligand or antibody. Such an assay is exemplified by the binding of tTF to target cells using the B21-2/10H10 bispecific antibody, as described in Example II.

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This culturing provides a population of hybridomas from which specific hybridomas are selected. Typically, selection of hybridomas is performed by culturing the cells by single-clone dilution in microtiter plates, followed by testing the individual clonal supernatants (after about two to three weeks) for the desired reactivity. The assay should be sensitive, simple and rapid, such as radioimmunoassays, enzyme immunoassays, cytotoxicity assays, plaque assays, dot immunobinding assays, and the like.

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☐ 6. Document ID: US 5877289 A

L5: Entry 6 of 7 File: USPT Mar 2, 1999

DOCUMENT-IDENTIFIER: US 5877289 A
TITLE: Tissue factor compositions and ligands for the specific coagulation of vasculature

DRPR:
FIG. 3A, FIG. 3B, FIG. 3C and FIG. 3D. Time course of vascular thrombosis and tumor necrosis after administration of coaguligand. Groups of 3 mice bearing 0.8 cm diameter C1300 (Mu.gamma.) tumors were given an intravenous injection of a coaguligand composed of 14 .mu.g B21-2/10H10 and 11 .mu.g tTF. FIG. 3A; Before injection: blood vessels are intact and tumor cells are healthy. FIG. 3B; 0.5 hours: blood vessels throughout the tumor are thrombosed; tumor cells are healthy. FIG. 3C; 4 hours: dense thrombi are present in all tumor vessels and tumor cells are separating and developing pyknotic nuclei. Erythrocytes are visible in the tumor interstitium. FIG. 3D; 24 hours: advanced tumor necrosis throughout the tumor. Arrows indicate blood vessels.

DRPR:
FIG. 4. Solid tumor regression induced by tumor-vasculature directed coaguligand therapy. Nu/nu mice bearing approximately 0.8 cm diameter C1300 (Mu.gamma.) tumors were given two intravenous injections of B21-2/10H10 (14 .mu.g) mixed with tTF (11 .mu.g) spaced 1 week apart (arrows) (.quadrature.). Mice in control groups received equivalent doses of tTF alone (.circle-solid.), B21-2/10H10 alone (.smallcircle.) or diluent (.box-solid.). Other control groups which received equivalent doses of isotype-matched control bispecific antibodies (SFR8/10H10, OX7/10H10 or B21-2/OX7) and tTF had similar tumor responses to those in animals receiving tTF alone. The number of mice per group was 7 or 8.

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The assay may be any one of a range of immunological assays based upon antibody binding and the TEC-4 or TEC-11 antibodies would be detected by means of

detecting their label, e.g., using streptavidin in the case of biotinylated antibodies or by using a chromogenic substrate in connection with an enzymatic label or by simply detecting the radiolabel. An antibody that binds to the same epitope as TEC-4 or TEC-11 will be able to effectively compete for binding and thus will significantly reduce TEC-4 or TEC-11 binding, as evidenced by a reduction in labelled antibody binding. In the present case, after mixing the labelled TEC-4 or TEC-11 antibodies with the test antibodies, suitable assays to determine the remaining reactivity include, e.g., ELISAs, RIAs or western blots using human endoglin; immunoprecipitation of endoglin; ELISAs, RIAs or immunofluorescent staining of recombinant cells expressing human endoglin; indirect immunofluorescent staining of tumor vasculature endothelial cells; reactivity with HUVEC or TCM-activated HUVEC cell surface determinants indirect immunofluorescence and FACS analysis. This latter method is most preferred and was employed to show that the epitopes recognized by TEC-4 and TEC-11 are distinct from that of 44G4 (Gougos & Letarte, 1988).

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The present studies show that soluble human tTF, possessing practically no ability to induce coagulation, became a powerful thrombogen for tumor vasculature when targeted by means of a bispecific antibody to tumor endothelial cells. In vitro coagulation studies showed that the restoration of thrombotic activity of tTF is mediated through its cross-linking to antigens on the cell surface.

DEPR:
Administration of a coaguligand directed against class II to mice having tumors with class II-expressing vasculature caused rapid thrombosis of blood vessels throughout the tumor. This was followed by infarction of the tumor and complete tumor regressions in a majority of animals. In those animals where complete regressions were not obtained, the tumors grew back from a surviving rim of tumor cells on the periphery of the tumor where it had infiltrated into the surrounding normal tissues. The vessels at the growing edge of the tumor lacked class II antigens, thus explaining the lack of thrombosis of these vessels by the coaguligand. It is likely that these surviving cells would have been killed by coadministering a drug acting on the tumor cells themselves, as was found previously (Burrows & Thorpe, 1993).

DEPR:
The anti-tumor effects of the coaguligand were similar in magnitude to those obtained in the same tumor model with an immunotoxin composed of anti-class II antibody and deglycosylated ricin A-chain (Burrows & Thorpe, 1993). One difference between the two agents is their rapidity of action. The coaguligand induced thrombosis of tumor vessels in less than 30 minutes whereas the immunotoxin took 6 hours to achieve the same effect. The immunotoxin acts more slowly because thrombosis is secondary to endothelial cell damage caused by the shutting down of protein syntheses.

DEPR:
A second and important difference between the immunotoxin and the coaguligand is that they have different toxic side effects. The immunotoxin caused a lethal destruction of class II-expressing gastrointestinal epithelium unless antibiotics were given to suppress class II induction by intestinal bacteria. The coaguligand caused no gastrointestinal damage, as expected because of the absence of clotting factors outside of the blood, but caused coagulopathies in occasional mice when administered at high dosage.

DEPR:
It is contemplated that patients chosen for the study would have failed to respond to at least one course of conventional therapy and had to have objectively measurable disease as determined by physical examination, laboratory techniques, or radiographic procedures. Where murine monoclonal antibody portions are employed, the patients should have no history of allergy to mouse immunoglobulin. Any chemotherapy should be stopped at least 2 weeks before entry into the study.

DEPR:
Physical examination, tumor measurements, and laboratory tests should, of course, be performed before treatment and at intervals up to 1 month later. Laboratory tests should include complete blood counts, serum creatinine, creatine kinase, electrolytes; urea, nitrogen, SGOT, bilirubin, albumin, and total serum protein. Serum samples taken up to 60 days after treatment should be evaluated by radioimmunoassay for the presence of the intact bispecific ligand or components thereof and antibodies against any or both portions of the ligand. Immunological analyses of sera, using any standard assay such as, for example, an ELISA or RIA, will allow the pharmacokinetics and clearance of the therapeutic agent to be evaluated.

DEPL:
3. Radiolabeling of Proteins

DEPU:
Griffin et al., Proc. 2nd Conf. on Radioimmunodetection & Therapy of Cancer, 82,

1988a.

DEPU:
Murray et al., Radio. Onc., 16:221-234, 1989.

DEPU:
Wawrzynczak & Thorpe, "Methods for preparing immunotoxins: effect of the linkage on activity and stability", in: Immunoconjugates,: Antibody conjugates in radioimaging and therapy of cancer, Vogel (ed), New York, Oxfod University Press, pp. 28-55, 1987.

ORPL:
Epenetos et al., "Limitations of Radiolabeled Monoclonal Antibodies for Localization of Human Neoplasms," Cancer Res., 46:3183-3191, 1986.

☐ 7. Document ID: US 5421832 A

L5: Entry 7 of 7 File: USPT Jun 6, 1995

DOCUMENT-IDENTIFIER: US 5421832 A
TITLE: Filter-catheter and method of manufacturing same

ABPL:
This invention relates to a filter for the partial and at least temporary interruption of a vein, comprising a tube made of polytetrafluoroethylene presenting towards its distal end longitudinal notches distributed symmetrically over its periphery and defining flexible bands, and a conduit made of polytetrafluoroethylene inside the tube; the conduit and the tube are joined for example by thermo-welding by their distal ends. A ring made of a radio-opaque material is preferably included between the distal ends during thermo-welding. The flexible bands open out transversely by the conduit sliding in the tube. They have undergone a treatment of thermo-setting, memorizing in the polymeric structure of the tetrafluoroethylene the opened out form and/or a helical form.

BSPR:
It is known that polytetrafluoroethylene is a material which is only very slightly thrombogen. However, the invention's merit lies in the fact that this material has been chosen as presenting a proven sufficient flexibility to effect transverse expansion of the flexible bands pre-cut-out on its periphery.

BSPR:
However, it is desirable to be able to follow the penetration and placing of the catheter inside the vein. To that end, it is known to employ a radio-opaque element near the filtering element. In the present case, the filter-catheter comprises a ring made of a radio-opaque material, for example gold, which is included between the tube and the inner conduit and fixed thereto during thermo-welding.

BSPR:
In this way, this radio-opaque element is not visible and cannot be a source either of catching on the wall of the vein nor of possible formation of blood clots.

DEPR:
The distal ends, 2a of tube 2 and 3a of conduit 3, respectively, are thermo-welded together and around a metal ring 4, made of stainless steel, platinum or gold. This ring 4 is made of a radio-opaque material, detectable during positioning of the filter.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Drawi Desc | Image

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☐ 1. Document ID: US 6147060 A

L4: Entry 1 of 17 File: USPT Nov 14, 2000

DOCUMENT-IDENTIFIER: US 6147060 A
TITLE: Treatment of carcinomas using squalamine in combination with other anti-cancer agents

DEPR:
The NHE antiporter of cells may be activated in different ways. For example, insoluble fibronectin activates the NHE antiporter by clustering and immobilizing Integrin .alpha..sub.v .beta..sub.1, independent of the cell shape (the growth of anchorage-dependent cells requires both soluble mitogens and insoluble matrix molecules). In addition, the attachment of stimuli to the extracellular matrix or cell attachment events involving viruses also activate the NHE antiporter.

DEPR:
FIG. 8 shows a graphical representation of the results of the rabbit cornea micropocket assay test. To provide a quantitative evaluation, the Angiogenesis Index ("AI") of each eye was determined. To determine the Angiogenesis Index, first the vessel density ("D.sub.vessel ") in an eye was graded on a 0-3 scale as follows:

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw. Desc | Image

☐ 2. Document ID: US 6130231 A

L4: Entry 2 of 17 File: USPT Oct 10, 2000

DOCUMENT-IDENTIFIER: US 6130231 A
TITLE: Integrin receptor antagonists

BSPR:
Angiogenesis or neovascularization is critical for normal physiological processes such as embryonic development and wound repair (Folkman and Shing, J. Biol. Chem. 1992, 267:10931-10934; D'Amore and Thompson, Ann. Rev. Physiol. 1987, 49:453-464). However, angiogenesis occurs pathologically, for example, in ocular neovascularization (leading to diabetic retinopathy, neovascular glaucoma, retinal vein occlusion and blindness), in rheumatoid arthritis and in solid tumors (Folkman and Shing, J. Biol. Chem., 1992, 267:10931-10934; Blood and Zetter, Biochim. Biophys. Acta 1990, 1032:118-128).

BSPR:
During endothelium injury, the basement membrane zones of blood vessels express several adhesive proteins, including but not limited to von Willebrand factor, fibronectin, and fibrin. Additionally, several members of the integrin family of adhesion receptors are expressed on the surface of endothelial, smooth muscle and on other circulating cells. Among these integrins is .alpha..sub.v .beta..sub.3, the endothelial cell, fibroblast, and smooth muscle cell receptor for adhesive proteins including von Willebrand factor, fibrinogen (fibrin), vitronectin, thrombospondin, and osteopontin. These integrins initiate a calcium-dependent signaling pathway that can lead to endothelial cell, smooth muscle cell migration and, therefore, may play a fundamental role in vascular cell biology.

BSPR:
The .alpha..sub.v .beta..sub.3 heterodimer is a member of the .beta..sub.3 integrin subfamily and has been identified on platelets, endothelial cells, melanoma, smooth muscle cells, and osteoclasts (Horton and Davies, J. Bone Min. Res. 1989, 4:803-808; Davies, et al., J. Cell. Biol. 1989, 109:1817-1826; Horton, Int. J. Exp. Pathol. 1990, 71:741-759). Like GPIIb/IIIa, the vitronectin receptor binds a variety of RGD-containing adhesive proteins such as vitronectin, fibronectin, VWF, fibrinogen, osteopontin, bone sialo protein II and thrombospondin in a manner mediated by the RGD sequence. A key event in bone resorption is the adhesion of osteoclasts to the matrix of bone. Studies with monoclonal antibodies have implicated the .alpha..sub.v .beta..sub.3 receptor in this process and suggest that a selective .alpha..sub.v .beta..sub.3 antagonist would have utility in blocking bone resorption (Horton, et al., J. Bone Miner. Res. 1993, 8:239-247; Helfrich, et al., J. Bone Miner. Res. 1992, 7:335-343)

DEPR:
The compounds of Formula I-IV have the ability to suppress/inhibit angiogenesis in vivo, for example, as demonstrated using animal models of ocular neovascularization.

☐ 3. Document ID: US 6130071 A

L4: Entry 3 of 17 File: USPT Oct 10, 2000

DOCUMENT-IDENTIFIER: US 6130071 A
TITLE: Vascular endothelial growth factor C (VEGF-C) .DELTA.Cys.sub.156 protein and gene, and uses thereof

BSPR:
For example, the biological effects of VEGF-C on vascular endothelial cells indicate in vivo uses for polypeptides of the invention for stimulating angiogenesis (e.g., during wound healing, in tissue transplantation, in eye diseases, in the formation of collateral vessels around arterial stenoses and into injured tissues after infarction) and for inhibiting angiogenesis (e.g., to inhibit tumor growth and/or metastatic cancer). The biological effects on vascular endothelial cells indicate in vitro uses for biologically active forms of VEGF-C to promote the growth of cultured vascular endothelial cells.

DRPR:
FIG. 1 schematically depicts major endothelial cell receptor tyrosine kinases and growth factors involved in vasculogenesis and angiogenesis. Major structural domains are depicted, including immunoglobulin-like domains (IGH), epidermal growth factor homology domains (EGFH), fibronectin type III domains (FNIII), transmembrane (TM) and juxtamembrane (JM) domains, tyrosine kinase (TK1, TK2) domains, kinase insert domains (KI), and carboxy-terminal domains (CT).

DEPR:
The expression pattern of the VEGFR-3 (Kaipainen et al., Proc. Natl. Acad. Sci. (USA), 92:3566-70 (1995)) suggests that VEGF-C may function in the formation of the venous and lymphatic vascular systems during embryogenesis. Constitutive expression of VEGF-C in adult tissues shown herein further suggests that this gene product also is involved in the maintenance of the differentiated functions of the lymphatic and certain venous endothelia where VEGFR-3 is expressed (Kaipainen et al., 1995). Lymphatic capillaries do not have well-formed basal laminae and an interesting possibility exists that the silk-like BR3P motif is involved in producing a supramolecular structure which could regulate the availability of VEGF-C in tissues. However, as shown here, VEGF-C also activates VEGFR-2, which is abundant in proliferating endothelial cells of vascular sprouts and branching vessels of embryonic tissues, but not so abundant in adult tissues. Millauer et al., Nature, 367:576-78 (1993). These data have suggested that VEGFR-2 is a major regulator of vasculogenesis and angiogenesis. VEGF-C may thus have a unique effect on lymphatic endothelium and a more redundant function, shared with VEGF, in angiogenesis and possibly in regulating the permeability of several types of endothelia. Because VEGF-C stimulates VEGFR-2 and promotes endothelial migration, VEGF-C may be useful as an inducer of angiogenesis of blood and lymphatic vessels in wound healing, in tissue transplantation, in eye diseases, and in the formation of collateral vessels around arterial stenoses and into injured tissues after infarction.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 4. Document ID: US 6090814 A

L4: Entry 4 of 17 File: USPT Jul 18, 2000

DOCUMENT-IDENTIFIER: US 6090814 A
TITLE: Quinazolinone-containing pharmaceutical compositions for prevention of neovascularization

BSPR:
Degradation and remodelling of the ECM are essential processes for angiogenesis. In addition, ECM components synthesized by endothelial cells (i.e., collagens, laminin, thrombospondin, fibronectin and SPARC) function to regulate endothelial cell growth, migration and shape [J. Bischoff, "Approaches to Studying Cell Adhesion Molecules in Angiogenesis," Trends Cell Biol., No. 5, pp. 69-74 (1995)]. It was reported that bovine aortic endothelial cells (BAE) undergoing sprouting and tube formation synthesize type I collagen and SPARC. It was proposed that type I collagen may be involved in directing migration and assembly of the BAE cells [M. L. Iruela-Arispe, et al., Lab. Invest., No. 64, pp. 174-186 (1991)]. It was also found that exogenous type I collagen promoted rapid tube formation by confluent human dermal microvascular endothelial cells [C. J. Jackson and K. L. Jenkins, Exp. Cell Res., No. 192, pp. 319-323 (1991)]. The tubes contained collagen fibrils in the luminal spaces, suggesting that the endothelial cells use the fibrids to fold and align into tube structures.

BSPR:
Protracted angiogenesis is observed in a variety of pathologic states, such as arthritis, psoriasis, diabetic retinopathy, chronic inflammation, scleroderma, hemangioma, retrolental fibroplasia and abnormal capillary proliferation in hemophiliac joints, prolonged menstruation and bleeding, and other disorders of the female reproductive system [J. Folkman, ibid. (1995); J. W. Miller, et al., "Vascular Endothelial Growth Factor/Vascular Permeability Factor Is Temporarily and Partially Correlated with Ocular Angiogenesis in a Primate Model," J. Pathol., Vol. 145, pp. 574-584 (1994); A. P. Adamis, et al., "Increased Vascular Endothelial Growth Factor Levels in the Vitreous of Eyes with Proliferative Diabetic Retinopathy," Amer. J. Ophthal., Vol. 118, pp. 445-450 (1994); K. Takahashi, et al., "Cellular Markers that Distinguish the Phases of Hemangioma during Infancy and Childhood," J. Clin. Invest., Vol. 93, pp. 2357-2364 (1994); D. J. Peacock, et al., "Angiogenesis Inhibition Suppresses Collagen Arthritis," J. Exp. Med., Vol. 175, pp. 1135-1138 (1992); B. J. Nickoloff, et al., "Aberrant Production of Interleukin-8 and Thrombospondin-1 by Psoriatic Keratinocytes Mediates Angiogenesis," Amer. J. Pathol., Vol. 44, pp. 820-828 (1994); J. Folkman, "Angiogenesis in Female Reproductive Organs," in: Steroid Hormones and Uterine Bleeding, N. J. Alexander and C. d'Arcangues, Eds., American Association for the Advancement of Science Press, Washington, D.C., U.S.A., pp. 144-158 (1992)].

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

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☐ 5. Document ID: US 6043218 A

L4: Entry 5 of 17 File: USPT Mar 28, 2000

DOCUMENT-IDENTIFIER: US 6043218 A
TITLE: Positively charged non-natural amino acids, methods of making thereof, and use thereof in peptides

BSPR:
Fibronectin, fibribnopeptide inhibitors and analogs, agonists and antagonists can be used to treat metastasis (i.e. enzyme inhibition, tumor cell migration, invasion, and metastasis).

BSPR:
Laminin-derived synthetic peptides analogs, agonists and antagonists can be used to treat tumor cell growth, angiogenesis, regeneration studies, vascularization of the eye with diabetes, and ischemia. Examples of these peptides are described in Kleinman, H. K.; Weeks, B. S.; Schnaper, H. W.; Kibbey, M. C.; Yamamura, K.; Grant, D. S; The laminins: a family of basement membrane glycoproteins important in cell differentiation and tumor metastases. Vitamins & Hormones. 47:161-86, 1993.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 6. Document ID: US 6028075 A

L4: Entry 6 of 17 File: USPT Feb 22, 2000

DOCUMENT-IDENTIFIER: US 6028075 A
TITLE: Quinazolinone containing pharmaceutical compositions for prevention of neovascularization and for treating malignancies

BSPR:
Degradation and remodeling of the ECM are essential processes for the mechanism of angiogenesis. In addition, ECM components synthesized by endothelial cells (i.e., collagens, laminin, thrombospondin, fibronectin and SPARC) function to regulate endothelial cell growth, migration and shape [J. Bischoff, Trends Cell Biol., No. 5, pp. 69-74 (1995)]. Bovine aortic endothelial cells (BAE) undergoing sprouting and tube formation synthesize type I collagen and SPARC. It was proposed that type I collagen may be involved in directing migration and assembly of the BAE cells [M. L. Iruela-Arispe, et al., Lab. Invest., No. 64, pp. 174-186 (1991). It was also found that exogenous type I collagen promoted rapid tube formation by confluent human dermal microvascular endothelial cells [C. J. Jackson and K. L. Jenkins, Exp. Cell Res., No. 192, pp. 319-323 (1991)]. The tubes contained collagen fibrils in the luminal spaces, suggesting that the endothelial cells use the fibrils to fold and align into tube structures.

ORPL:
Miller et al "Vascular Endothelial Growth factor/Vascular Permeability factor is tempoarilyand Spatially Correlated with Ocular Angiogenesis in a Primate Model", Am. J. Path., vol. 145, No. 3, pp. 574-584 (1994).

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 7. Document ID: US 5994388 A

L4: Entry 7 of 17 File: USPT Nov 30, 1999